Method for isolating nucleic acids

The invention relates to a method for separating off and selectively liberating nucleic acids by use of special bead polymers

So-called genetic diagnosis has become increasingly important recently.
Genetic diagnosis has become involved in the diagnosis of human diseases (inter alia detection of pathogens, detection of genome mutations, analysis of circulating tumor cells and identification of risk factors for predisposition to a disease). However, genetic diagnosis is also now finding applications in veterinary medicine, environmental analysis and food testing. A further area of application comprises investigations in institutes of pathology/cytology or within the framework of forensic problems. However, genetic diagnosis is now employed also for the purposes of quality and process control (for example investigations of blood samples for freedom from pathogens), and legislation is planned to extend the number of tests already required now by law in the future.

Methods also employed in genetic diagnosis (such as, for example, hybridization and amplification techniques such as PCR, bDNA or NASBA, TMA technology) are also among the routine methods in fundamental scientific studies.

The increasingly wide use of nonradioactive detection methods, which also play a part in genetic diagnosis, leads to the expectation that genetic diagnosis will be used even more widely in future than at present.

An important step in genetic diagnosis is the obtaining of gene samples from biological materials such as cells, blood, sputum, CSF, serum or urine.

The binding of nucleic acids to polyquaternary cationic polymers is disclosed in US-A-4 046 750. However, the binding is irreversible, so that the adsorbed nucleic acids cannot be liberated again in this method.

US-A-4 055 469 discloses a method for purifying enzymes where nucleic acids and unwanted proteins are precipitated with the aid of water-soluble cationic polymers.

US-A-4 839 231 discloses supports which are coated with vinylpyridine polymer and are able to adsorb proteins and nucleic acids. However, the capacity of the supports is rather low and the adsorption of the nucleic acids is not quantitative.

WO-A-91/05606 describes a silanized, porous support material with hydroxyalkylamino groups for chromatographic separation of nucleic acids. However, this material is less well suited to rapid and maximally quantitative separation of nucleic acids out of biological materials.

DE-A-4 139 664 describes an apparatus and a method for isolating and purifying nucleic acids with the aid of anion exchangers. The disadvantages of this method are that it is possible to desorb the nucleic acids off the anion exchanger only with buffer solutions of high ionic strength, and the separation of the salts out of the buffer solution requires additional preparation steps.

DE-A-4 333 805 claims the extraction of nucleic acids from a sample with the aid of water-soluble carriers such as dextran, acrylamide or carboxymethylcellulose and other reagents, with the nucleic acids being precipitated.

EP-A-0 707 077 (corresponds to US-A-5 582 988) describes a method for isolating nucleic acids from biological material using soluble, weakly basic polymer. In this method, a precipitate is generated from a soluble, weakly basic polymer and the nucleic acid in an acidic pH range, the precipitate is separated from the

unprecipitated constituents of the biological material, and washed, and the nucleic acid is liberated again from the precipitate by adjusting a basic pH.

One disadvantage of the methods of DE-A-4 333 805 and EP-A-0 707 077 is that the manipulation, in particular the separation and purification of the precipitate, is difficult and very time consuming. These methods can moreover be carried out using automatic analyzers only under difficult conditions or not at all.

WO-A-96/18731 describes a method for isolating nucleic acids with the aid of a detergent and of a solid support. Since solid supports without pores and without swellability are employed, the binding capacity of the supports is relatively low.

WO-A-97/08547 describes a method for isolating nucleic acids in which the nucleic acids are bound to a solid hydrophilic organic polymer without effective positive charge, for example to a cellulose. In this method, the binding is effected by weak forces such as Van der Waals interactions.

WO-A-97/34909 describes a method for isolating nucleic acids by use of a specific particulate polymer with a lower critical solubility temperature (LCST) of 25-45° C. A disadvantage of this method is that buffers of high ionic strength must be used to liberate the nucleic acids but may interfere with further use of the nucleic acids. Because of the small size of the particles used, from 0.05 to 2 µm, moreover, the processing is time-consuming and difficult to automate.

Despite the numerous previously described methods for isolating nucleic acids, there is still a pressing need for a simple method, which can be automated if possible, for the effective separation of nucleic acids from biological material, and the liberation again thereof.

It has now been found, surprisingly, that certain water-insoluble bead polymers of polymerized units of amino monomer, crosslinker and vinyl monomer are outstandingly suitable for isolating nucleic acids.

The invention relates to a method for isolating nucleic acids from a sample, comprising the following steps

A) mixing the sample with a water-insoluble polymer which is not ionic in the basic and neutral range, at a pH of 7 or less, with the nucleic acids being adsorbed,

B) separating off the water-insoluble polymer and

C) mixing the water-insoluble polymer with an aqueous phase with a pH of greater than 7, with the adsorbed nucleic acids being liberated,

characterized in that the water-insoluble polymer is a bead polymer with an average particle size of from 3 to 100 µm and consists of polymerized units of

a) 5 to 98% by weight of amino monomer
b) 0.3 to 30% by weight of crosslinker and
c) 0 to 93% by weight of vinyl monomer.

Where appropriate, in a step interpolated after method step A) the biological material is lysed.

The present invention preferably relates to a method for isolating nucleic acids from a sample, comprising the steps

A) mixing the sample with a water-insoluble polymer which is not ionic in the basic and neutral range, at a pH of 7 or less, with the nucleic acids being adsorbed,

B) separating off the water-insoluble polymer and

C) mixing the water-insoluble polymer with an aqueous phase with a pH of greater than 7, with the adsorbed nucleic acids being liberated,

characterized in that the water-insoluble polymer is a bead polymer with an average particle size of from 3 to 100 µm and a specific surface area measured by the BET method of from 5 to 500 m^2/g and consists of polymerized units of

a) 5 to 98% by weight of amino monomer
b) 0.3 to 30% by weight of crosslinker and
c1) 0 to 93% by weight of hydrophobic vinyl monomer

or in that the water-insoluble polymer consists of

bead polymer which is able to swell in water well and has an average particle size of from 3 to 100 µm, and which consists of polymerized units of

a) 5 to 79.7% by weight of amino monomer
b) 0.3 to 10% by weight of crosslinker and
c2) 10 to 93% by weight of hydrophilic vinyl monomer.

The present invention particularly preferably relates to a method for isolating nucleic acids from a sample, comprising steps A), B) and C) defined above, characterized in that the water-insoluble polymer is a macroporous bead polymer with a particle size of from 3 to 100 µm and a pore diameter in the range from 10 to 1 000 nm, which

has a specific surface area determined by the BET method of 5 to 500 m^2/g (very particularly preferably 20 to 200 m^2/g) and consists of polymerized units of

a) 5 to 98% by weight of amino monomer
b) 2 to 30% by weight of crosslinker and
c1) 0 to 93% by weight of hydrophobic vinyl monomer

or in that the water-insoluble polymer consists of

bead polymer which is able to swell in water well and has an average particle size of from 3 to 100 µm, and which consists of polymerized units of

a) 5 to 79.7% by weight of amino monomer
b) 0.3 to 10% by weight of crosslinker and
c2) 10 to 93% by weight of hydrophilic vinyl monomer.

The method of the invention is suitable for the isolation and/or purification of nucleic acids of varying origin, for example from cells, tissue materials, blood or pathogens. Before the isolation of the nucleic acids, the material to be investigated is disrupted by techniques known per se, such as, for example, disruption by protease digestion, resulting in a sample, a lysate, suitable for subsequent steps A to C. Where appropriate, the biological material is lysed in a step interpolated after method step A). Further suitable disruption methods have been described in DE-A-4 333 805.

The sample is mixed with a water-insoluble polymer at a pH of 7 or less, preferably in the range 2 – 6, particularly preferably in the range 2 – 3, at room temperature. The water-insoluble polymer is separated off for example by filtration or by centrifugation. The complex of nucleic acid and polymer which is obtained in this way can then be purified by washing with suitable buffers such as, for example, TE.

To liberate the bound nucleic acids from the complex, the pH of the complex is then adjusted to pH values above 7, preferably of 8 – 14, particularly preferably in the range 12 – 14.

The bead polymers of the invention provide higher rates of adsorption and liberation again than the soluble polymers disclosed in EP-A-0 707 077. The isolation can be carried out more easily, i.e. with fewer working steps and in shorter times. The purity of the isolated nucleic acids is higher and, in particular, they contain fewer inhibiting byproducts so that amplification of the nucleic acids, for example by the so-called "PCR reaction" and the "RT-PCR", takes place particularly well. The method of the invention is also superior to the method described in EP-A-0 707 077 in relation to restriction enzyme digestion of the nucleic acids obtained.

The present invention further relates to the macroporous bead polymers, characterized in that they have an average particle size of from 3 to 100 μm, a pore diameter of from 10 to 1000 nm and a specific surface area measured by the BET method of from 5 to 500 m^2/g and consist of polymerized units of

a) 5 to 98% by weight of amino monomer
b) 2 to 30% by weight of crosslinker and
c1) 0 to 93% by weight of hydrophobic vinyl monomer,

and

the bead polymers which are insoluble but swellable in water, characterized in that they have an average particle size of from 3 to 100 μm and consist of polymerized units of

a) 5 to 79.7% by weight of amino monomer
b) 0.3 to 10% by weight of crosslinker and
c1) 10 to 93% by weight of hydrophilic vinyl monomer.

The present invention further relates to a method for preparing water-insoluble, macroporous bead polymers with an average particle size of from 3 to 100 µm, a pore diameter of from 10 to 1000 nm and a specific surface area measured by the BET method of from 5 to 500 m^2/g, characterized in that a mixture of

a) 5 to 98 parts by weight of amino monomer
b) 2 to 30 parts by weight of crosslinker
c1) 0 to 93 parts by weight of hydrophobic vinyl monomer
d) 10 - 150 parts by weight of porogen and
e) 0.1 – 2.5 parts by weight of free-radical former

is dispersed in an aqueous medium using a protective colloid, subsequently the resulting dispersion is polymerized by heating to the decomposition temperature of the free-radical former and, after the polymerization has taken place, the porogen is removed by extraction and/or evaporation,

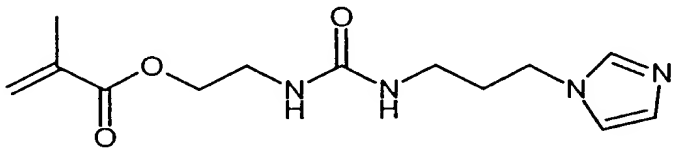
and a method for preparing bead polymers which are insoluble but swellable in water and have an average particle size of from 3 to 100 µm, characterized in that a mixture of

a) 5 to 79.7% by weight of amino monomer
b) 0.3 to 10% by weight of crosslinker and
c2) 10 to 93% by weight of hydrophilic vinyl monomer
d) 10-150 parts by weight of solvent and
e) 0.1 – 2.5 parts by weight of free-radical former

is dispersed in an aqueous medium using a protective colloid, subsequently the resulting dispersion is polymerized by heating to the decomposition temperature of the free-radical former and, after polymerization has taken place, the solvent is removed by extraction and/or evaporation.

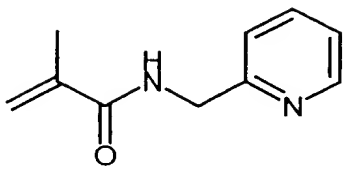
Amino monomers (a) for the purposes of the invention are polymerizable, ethylenically unsaturated compounds with at least one primary, secondary or tertiary amino group. The secondary or tertiary amino group may moreover be part of a cycloaliphatic or aromatic ring. Examples which may be mentioned are N-vinylimidazole, N-vinylbenzimidazole, 2-vinylpyridine and 4-vinylpyridine. Very suitable amino monomers are also the derivatives of acrylic acid and methacrylic acid, such as, for example, 2-aminoethyl methacrylate, N,N-dimethylaminoethyl methacrylate, N,N-dimethylaminopropyl methacrylate, N,N-dimethylaminoethyl acrylate, N-tert-butylaminopropyl methacrylate, N-(3-aminopropyl)methacrylamide, N-(3-imidazoylpropyl)methacrylamide, N-(2-imidazoylethyl)methacrylamide, N-(3-aminopropyl)acrylamide, N-(3-imidazoylpropyl)acrylamide, N-(2-imidazoylethyl)acrylamide, N-(1,1-dimethyl-3-imidazoylpropyl)methacrylamide, N-(1,1-Dimethyl-3-imidazoylpropyl)acrylamide, N-(3-benzimidazoylpropyl)methacrylamide and (3-benzimidazoylpropyl)acrylamide.

Very suitable amino monomers are also the products of the reaction of isocyanatoethyl (meth)acrylate and imidazoylalkylamines, such as, for example, the amino monomer of formula (I) which is novel within the framework of the present invention



(I)

Further suitable amino monomers according to the present invention are the pyridine derivatives of the formulae (II) and (III)



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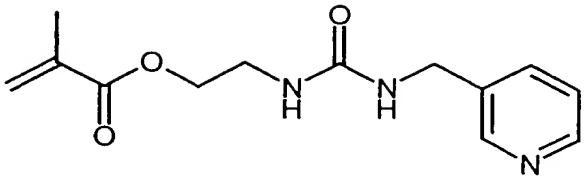

(III).

Derivatives of styrene and α-methylstyrene with amino groups are also very suitable. Examples which may be mentioned are: 4-N,N-dimethylaminostyrene, 2-N,N-dimethylaminostyrene. 4-N,N-diethylaminostyrene and 4-N,N-bis(2-hydroethyl)aminostyrene.

Crosslinkers (b) suitable according to the present invention are: ethylene glycol dimethacrylate, butanediol dimethacrylate, hexanediol dimethacrylate, pentaerythritol dimethacrylate, glycerol 1,2-dimethacrylate, glycerol 1,3-dimethacrylate, triethylene glycol dimethacrylate, tetraethylene glycol dimethacrylate, trimethylolpropane trimethacrylate, pentaerythritol trimethacrylate, pentaerythritol tetramethacrylate, ethylene glycol diacrylate, butanediol diacrylate, pentaerythritol diacrylate, glycerol 1,3-diacrylate, triethylene glycol diacrylate, trimethylolpropane triacrylate, pentaerythritol triacrylate, pentaerythritol tetraacrylate, allyl methacrylate, allyl acrylate, methylene-N,N'-bisacrylamide, p-divinylbenzene and m-divinylbenzene.

Hydrophobic vinyl monomers (c1) which may be present in the bead polymer of the invention and are suitable for the purposes of the present invention are C_1-C_8-alkyl acrylates, C_1-C_8-alkyl methacrylates, such as, for example, methyl methacrylate or butyl acrylate, acrylonitrile, methacrylonitrile, vinyl chloride, vinylidene chloride, vinyl acetate and aromatic vinyl monomers such as, for example, styrene, vinylnaphthalene, vinyltoluene, ethylstyrene, α-methylstyrene. chlorostyrenes and vinylbenzyl chloride.

Hydrophilic vinyl monomers (c2) suitable for the purposes of the present invention are, for example: 2-hydroxyethyl methacrylate, 2-hydroxypropyl methacrylate, 2-hydroxyethyl acrylate, 2-hydroxypropyl acrylate, triethylene glycol monomethacrylate, tetraethylene glycol monomethacrylate, acrylamide, methacrylamide and N,N-dimethylacrylamide.

Porogens are employed for the method of the invention for preparing water-insoluble macroporous bead polymers. Suitable for this purpose are liquid water-immiscible compounds which dissolve the monomers employed and precipitate the polymer formed. Examples which may be mentioned are aliphatic hydrocarbons such as hexane, heptane, octane, isooctane isododecane and alcohols such as octanol. The porogen is employed in amounts of from 10 to 150% by weight, preferably from 20 to 100% by weight, based on the total of the monomers and crosslinker employed.

Suitable and preferred free-radical formers for the purposes of the present invention are oil-soluble initiators. Examples which may be mentioned are: peroxy compounds such as dibenozoyl peroxide, dilauryl peroxide, bis (p-chlorobenzoyl peroxide), dicyclohexyl peroxydicarbonate, tert-butyl peroctoate, 2,5-bis(2-ethylhexanoylperoxy)-2,5-dimethylhexane and tert-amylperoxy-2-ethylhexane, in addition azo compounds such as 2,2′-azobis(isobutyronitrile), 2,2′-azobis(2,4-dimethylvalereonitrile) and 2,2′-azobis(2-methylisobutyronitrile). The initiators are generally used in amounts of from 0.05 to 2.5% by weight, preferably 0.2 to 1.5% by weight, based on the monomer mixture.

In the preparation of the macroporous gel bead polymers of the invention there is use, if appropriate, of protective colloids in the aqueous phase. Suitable protective colloids according to the present invention are natural and synthetic water-soluble polymers such as, for example, gelatin, starch, cellulose derivatives, in particular cellulose esters and cellulose ethers, polyvinyl alcohol, polyvinylpyrrolidone, polyacrylic acid, polymethacrylic acid and copolymers of acrylic acid, methacrylic acid, methacrylic esters and/or acrylic esters. Copolymers of methacrylic acid and

methacrylic ester neutralized with alkali metal hydroxide are particularly suitable. The amount of protective colloid employed is generally 0.05 to 2% based on the aqueous phase, preferably 0.1 to 1%.

The aqueous phase may additionally contain where appropriate a buffer system. Preferred buffer systems adjust the pH of the aqueous phase at the start of the polymerization to a value between 12 and 5, preferably between 10 and 6. Under these conditions, dispersants with carboxyl groups are wholly or partly in the form of salts. The effect of the protective colloids is beneficially influenced in this way. Particularly suitable buffer systems contain phosphate or borate salts.

The amount of the aqueous phase is generally 75 to 1200% by weight, preferably 100 to 500% by weight, based on the total of monomers, crosslinker and porogen.

The stirring speed during the polymerization is important for adjusting the particle size. In the preparation method of the invention the size of the resulting bead polymers decreases with increasing stirrer speed. The exact stirrer speed for adjusting a particular predetermined bead size depends in the individual case greatly on the size of the reactor, the reactor geometry and the stirrer geometry. It has proved to be expedient to determine the necessary stirring speed by experiment. For laboratory reactors with a reaction volume of 3 liters and equipped with paddle stirrers, on use of copolymers of acrylic acid, methacrylic acid, acrylic esters and/or methacrylic esters as dispersant in general bead sizes of from 6 to 30 µm are reached with speeds of from 300 to 500 rpm.

The polymerization temperature in the preparation method of the invention depends on the decomposition temperature of the initiator employed. It is generally between 50 and 150°C, preferably between 55 and 100°C. Polymerization takes 0.5 to some hours. It has proved appropriate to use a temperature program in which the polymerization is started at low temperature, for example 70°C, and the reaction temperature is raised as the polymerization reaction progresses.

After the polymerization it is possible for the polymer to be isolated by conventional methods, for example by filtration or decantation, where appropriate after one or more washes, to be dried. The porogen can be removed during the drying. In the case of low-boiling porogens such as, for example, hexane, it is also possible to remove the porogen from the aqueous reaction mixture wholly or partly by distillation before isolating the bead polymer.

Preparation of the bead polymers capable of swelling in water takes place in analogy to the preparation of the macroporous bead polymers, employing hydrophilic vinyl monomers (c2) in place of the hydrophobic vinyl monomers (c1), and a solvent in place of the porogen.

Suitable solids are those which are immiscible with water and dissolve the monomers and the crosslinker and do not precipitate, but dissolve or swell, the polymer formed. Suitable solvents are toluene, xylene, tetrachloromethane, chloroform, methylene chloride, dichloroethane and ethyl acetate. The amount of auxiliary solvent is generally 10 to 200% by weight, preferably 10 to 150% by weight, particularly preferably 20 to 100% by weight, based on the total of monomers and crosslinker. If required, the auxiliary solvent can be removed for example by distillation after the polymerization. Toluene can be removed particularly simply by azeotropic distillation.

The bead polymers capable of swelling in water according to the invention have swelling indices of from 1.2 to 12, preferably 1.5 to 8, measured at 25°C and pH 7. The swelling index is defined as the quotient of the volume of the bead polymer swollen to saturation in water and the volume of the anhydrous bead polymer.

The present application also relates to compositions for isolating nucleic acids comprising water-insoluble macroporous bead polymers with an average particle size of from 3 to 100 µm, a pore diameter of from 10 to 1000 nm and a specific surface

area measured by the BET method of from 5 to 500 m^2/g, consisting of polymerized units of

a) 5 to 98% by weight of amino monomer

b) 2 to 30% by weight of crosslinker

c1) 0 to 93% by weight of hydrophobic vinyl monomer

or of bead polymers which are insoluble but swellable in water and have an average particle size of from 3 to 100 µm, consisting of polymerized units of

a) 5 to 79.7% by weight of amino monomer

b) 0.3 to 10% by weight of crosslinker and

c2) 10 to 93% by weight of hydrophilic vinyl monomer.

These compositions are preferably in the form of aqueous dispersions with a solids content of 0.1 – 50%, particularly preferably 1 – 10%. The aqueous phase may, where appropriate, contain buffers which are preferably effective in the range 2 – 6.

Possible areas of use of a composition which is formulated, for example, as a test kit are the application examples already mentioned above, for example in the isolation of nucleic acids from cells, tissue materials, blood or pathogens, with all diagnostic questions in particular being involved. The described polymers are also understood as test kit for routine nucleic acid tests in microtiter plates and/or test tubes, or other formats such as, for example, within the framework of chip technology.

Examples

Example 1

Preparation of the amino monomer of the formula 1

62.08 g (0.4 mol) of 2-isocyanatoethyl methacrylate were added dropwise over the course of 60 min to a stirred solution of 50.07 g (0.4 mol) of 3-aminopropylimidazole, 140 mg of 2,6-di-tert.butyl-4-methylphenol (stabilizer), 140 mg of dibutyltin dilaurate (catalyst) in 250 ml of chloroform at 20°C with cooling. The mixture was then heated at 50°C until (about 5 h) no NCO band was detectable in the IR spectrum. 112 g of amino monomer of the formula (I) were obtained.

Example 2

Preparation of a gel bead polymer

A solution of 5 g of polyvinyl alcohol (Mowiol® 40-88) and 1.5 g of disodium hydrogen phosphate in 130 ml of deionized water was introduced into a 250 ml reaction vessel with paddle stirrer, reflux condenser, thermometer, gas inlet tube and gas outlet tube. To this aqueous solution was added, at 20°C while stirring at 450 rpm, an organic solution of 7.91 g of N,N-dimethylaminoethyl methacrylate, 7 g of 2-hydroxyethyl methacrylate, 0.17g of triethylene glycol dimethacrylate, 0.225 g of 2,2′-azobis(2,4-dimethylvalereonitrile) and 37.5 g of chloroform over the course of 15 min. The mixture was gently flushed with nitrogen, and the temperature was raised to 67°C and kept at this temperature for 20 hours. After cooling, the bead polymer which had formed was separated off from the reaction liquor by decantation and freed of chloroform in vacuo at 50°C. 13.5 g of bead polymer with an average particle size of 20 µm and a swelling index of 5.1 measured at 25° C in water were obtained.

Example 3

Preparation of a macroporous bead polymer

A solution of 42.5 g of polyvinyl alcohol (Moviol 40-88) and 12.75 g of disodium hydrogen phosphate in 1240 g of deionized water was introduced into a 2 liter reaction vessel with paddle stirrer, reflux condenser, thermometer, gas inlet tube and gas outlet tube. To this aqueous solution was added, at 20° C while stirring at 280 rpm (revolutions per minute), an organic solution of 23.71 g of N,N-dimethylaminoethyl methacrylate, 13.04 g of styrene, 10.67 g of divinylbenzene, 0.71g of 2,2'-azobis(2,4-dimethylvalereonitrile) and 31.62 g of hexane over the course of 30 min at 25°C. The mixture was gently flushed with nitrogen, and the temperature was raised to 66°C and kept at this temperature for 20 hours. The hexane was then distilled out at an internal temperature of 70 to 98° C. After cooling, the resulting bead polymer was separated from the reaction liquor by decantation and dried in vacuo at 50° C. 38 g of bead polymer with an average particle size of 15 µm and a specific surface area of 62.3 m^2/g were obtained.

Example 4

Preparation of a macroporous bead polymer

Example 3 was repeated employing an organic solution of 23.71 g of amino monomer from Example 1, 13.04 g of styrene, 10.67 g of divinylbenzene, 0.71g of 2,2'-azobis(2,4-dimethylvalereonitrile) and 38 g of hexane. 35 g of macroporous bead polymer with an average particle size of 25 µm and a specific surface area of 74 m^2/g were obtained.

Biological result

- 100 µl of blood were mixed with 10^5 Caski cells. These cells contain human papillomavirus of type 16 (HPV).
- 10 µl of a 2.4% strength particle dispersion from Example 2 were added to this mixture..
- After addition of 200 µl of a suitable buffer, for example TE, incubation took place at room temperature for 5 min (TE represents 10 mmol of tris HCl and 1 mmol of ethylenediaminetetraacetic acid pH 7.4 in the final concentration).
- The particles were then sedimented in an Eppendorf centrifuge at 7 000 rpm for 3 min.
- Then 200 µl of a suitable lysis buffer, for example 0.5% IGEPAL CA-630® in TE (IGEPAL CA-630® is a nonionic detergent which can be purchased, for example, through Sigma, order number I 3021), were added to the sediment and incubated anew at room temperature for 5 min.

  In place of IGEPAL CA-630® it is also possible, however, to employ other lysis buffers. Examples which may be mentioned are classical methods such as with protease K digestion and subsequent purification using phenol/chloroform or sodium lauryl sulfate solutions (Sigma order number: L 6026), for example as 0.5% strength aqueous solution.
- Renewed centrifugation and sedimentation took place in an Eppendorf centrifuge at 7000 rpm for 3 min.
- The particles were subsequently washed 2 x with a suitable buffer (for example TE). After the second washing step, the supernatant was discarded and further work was done only with the particles.
- The liberation of the nucleic acid bound to the particles took place by adjusting the pH to >12 by adding 1 µl of 0.5 normal NaOH.
- This was followed by incubation at room temperature for 15 minutes.
- After centrifugation (3 min, 7000 rpm in an Eppendorf centrifuge), the concentration of the nucleic acid obtained was determined by a suitable

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method, for example by analysis in a gel system, particularly preferably the "Submerged Gel Nucleic Acid Electrophoresis System", order No. 170 4406 from BIO-RAD (webpage: www.bio-rad.com).

Compared with beads for nucleic acid extraction known from the method of EP-A-0 707 077, distinctly better results were surprisingly achieved with the method of the invention and the bead polymers used therein.

- 15 µl of the supernatant obtained in this way were then employed in an HPV-specific PCR (polymerase chain reaction), that is to say primer was employed in a PCR which are specific for human papillomaviruses (HPV). On analysis of the result in a gel system it was possible to identify the expected nucleic acid band clearly and distinctly.